FABRICATION AND CHARACTERIZATION OF COMPOSITE BIODEGRADABLE FILMS FROM CHITOSAN AND CORN SILK

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"I declare that this thesis entitled *Fabrication and Characterization of Composite Biodegradable Film from Chitosan and Corn Silk* is the result of my own research except as cited in the references. The thesis has not been accepted for any degree and is not concurrently submitted in candidature of any other degree"

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ABSTRACT

Composite biodegradable film made from chitosan has been most potential type of packaging especially in food packaging. Corn silk and starch, were used to enhance the physical and chemical properties of the composite biodegradable film. Corn silk is good to increase physical properties because it consist of zein a corn protein. It also contain some of chemical compound that is good to fight the mycotoxins. The film preparation process consist of hydrolyzation of corn silk fibers which was done at a temperature range of 80°C before to be mixed with chitosan based solution. The solution was then casted and characterized in terms of morphology changes and physical and chemical properties. Based on the study, the fibers from corn silk do gives an extra physical properties for the chitosan-base composite film. The melting point has change to a more better value in order to sustain more heat during packaging before it gradually melts compared to chitosan alone. After the fiber being added with starch, the composite film able to give higher melting point Futhermore,the mechanical and thermal strength increase but only by small value. This is due to the effect of miscibility of solution before casting it to be a film. Results obtain from FTIR, TGA, DSC and AFM proved that by using corn silk as an additive, the composite film is experimentally enhanced in terms of physical properties. From the study, it can be concluded that the fibers from corn silk fiber has a great potential for being fiber to support the matrix base composite film but there must be further research on how to mix the fiber well with the starch.

ABSTRAK

Bio komposit filem yang mempunyai asas matriks dari bahan chitosan merupakan bahan untuk membungkus. Chitosan, bahan yang berjaya menarik perhatian ramai saintis dan pengkaji dengan kelebihan yang ada di mana chitosan merupakan bahan yang mempunyai sifat sifat fizikal yang sangat bagus untuk dijadikan filem serta anti-fungi yang ada dalam sifatnya yang membolehkan ia bebas dari bakteria yang berbahaya.Walaubagaimanapun, terdapat masalah di mana masih tiada bahan yang sangat sesuai dijadikan sebagai agen penambah tetapi bahan organik merupakan bahan yang paling sesuai kerana mempunyai fiber organik untuk mengukuhkan filem walaupun terdapat sebahagian sintetik fiber. Bulu jagung dan kanji merupakan bahan yang dalam kajian mampu menambah baikkan ciri ciri fizikal dan kimia di dalam komposit filem tersebut. Bulu jagung mempunyai ciri ciri yang baik dari segi fizikal di mana terdapat bahan zein protein yang merupakan protein jagung. Ia juga mempunyai bahan kimia yang baik untuk melawan bakteria ataupun mikotoksin Proses filem ini bermula dengan proses hidrolisis fiber bulu jagung yang di jalankan sekitar 80^{o}C sebelum dicampurkan dengan asas campuran cecair chitosan. Cecair itu kemudian dituang di atas gelas kaca dan dibiarkan kering selama satu hari. Dan dikaji dari segi permukaannya, ciri-ciri fizikal dan kimianya .Daripada kajian yang dijalankan, fiber bulu jagung berjaya memberi kesan yang lebih baik kepada ciri ciri fizikal komposit filem chitosan kerana ia mengubah takat lebur yang lebih baik daripada takat lebur biasa asas chitosan sahaja. Selepas ia di tambah pula dengan kanji, komposit memberi keputusan yang lebih baik tetapi dengan hanya sedikit perubahan. Ia disebabkan oleh kesan kebolehan bercampur. Keputusan yang diberikan oleh FTIR, TGA, DSC dan AFM membuktikan bulu jagung dan kanji mampu menambah baik ciri ciri fizikal .Daripada kajian yang dijalankan, bulu jagung dan kanji mampu menjadi fiber untuk menyokong struktur asas matrik dalam komposit biofilem tetapi masih memerlukan kajian untuk meningkatkan kebolehan bercampur antara bahan tersebut

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LIST OF ABBREVIATIONS

v/v	-	volume / volume
Mt	-	Metric Tonne
um	-	ultra meter
g	-	Gram
OH-	-	Hydroxide functional group
NH-	-	Amide functional group
PVA	-	Polyvinyl Alcohol
CGM	-	Corn Gluten Meal
AFM	-	Atomic Force Microscope
FTIR	-	Fourier Transform Infrared
TGA	-	Thermo Gravimetric Analysis
DSC	-	Differential Scanning Calorimeter
SEM	-	Scanning Electron Miscroscopy

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CHAPTER 1

INTRODUCTION

1.1 Background of Study

It is normally when the consumer products purchased in our everyday lives have either come with or been dispensed in packaging films or wrappers where its distinct role in contributing enormously to the food packaging industry alone is ascertained by the fact that these films are utilized to fulfill at least one of the least functions such as to provide product protection from physical damage, contamination and deterioration.

Other trends and demands that significantly influence food safety are documentation and traceability, legislation, and consumer demand for safer, healthier, more convenient,better tasting, lower cost and environmentally friendly products.

Particularly, the food packaging films help to further the extend food's shelf-life properties by colossally ward off oxidative and microbial spoilage. By that idea, its fair to sum up that the purpose of food packaging is to preserve the quality and safety of the food contained from the time of manufacture until the time it is used by the consumer (Cutter, 2006; Marsh and Bugusu, 2007).

Starkly notable to the food packaging industry are the emphasis and pressure being tossed upon effective food packaging technology. The use of protective films

or coatings and suitable packaging by the food industry has nevertheless switched mainstream and become an ongoing topic of monumental interest because of their packaging potentiality attributed to the ability in increasing the shelf life of many food products (Sorrentino *et al.*, 2007).

Furthermore, food packaging technologies putting antimicrobial packaging films at the forefront play a pivotal role since many food products are, most of the time, subjected to microbial attacks and contamination (Appendini and Hotchkiss, 2002) by undesirable microbes such as fungi, yeast and bacteria during the courses of production, packaging and distribution. Thus, it is essentially important to note that many novel packaging technologies are continually being developed to prolong the shelf-life and improve the safety or sensory properties of fresh foods.

By means of the correct selection of materials and packaging technologies, it is tantamount to the possibilities of being able to keep the product quality and freshness during the time required for its commercialization and most importantly, consumption (Stewart *et al.*, 2002).

In the mean time, the researches still keep going to find a new material to replace the non- biodegradeable material. Packaging technologies such as modified atmosphere packaging and antimicrobial active packaging based on volatile (essential oils and alcohols) or non-volatile components (chitosan and nisin) have been developed as a result of these demands (Betty- Cory , 2006)

Even though most of the packaging films used today to preserve foods are of synthetic origin, it is worth to say that in recent years, bio-based materials such as carbohydrates and proteins have, gradually if not extensively, been tested and experimented to develop biodegradable films which had been proven to have more and more versatile properties (Perez-Mateos *et al.*, 2007). This has undoubtedly set off and given rise to the diverse utilization of packaging films made of bio-based materials.

1.2 Problem Statement

Since the last 20 years, petrochemical polymers, commonly called “plastics,” have been booming and are by far the most widely used polymers for packaging because of their high performance and low cost (Callegarin *et al*., 1997). Until as recent as today, the largest part of all materials used in the packaging industries is produced from fossil fuels and practically non-biodegradable (Sorrentino *et al.*, 2007).

For this, the packaging materials for foodstuff, like any other short-term storage packaging material, represent a serious global environmental problem (Kirwan and Strawbridge, 2003). Normally these packaging stuff littered on the main road and being washed away by the rain through the drainage system and end up in the river.

A big effort to extend the shelf life and enhance food quality while reducing packaging waste has encouraged the exploration of new bio-based packaging materials, such as edible and biodegradable films from renewable resources (Tharanathan, 2003).

The goal of food packaging is to contain food in a cost-effective way that satisfies industry requirements and consumer desires, maintains food safety, and minimizes environmental impact (Marsh and Bugusu, 2007).

1.3 Significance of Study

Vast of interest in the search for biologically compound from natural sources has taken part in development of biodegradable compound in recent years. Some of the research able to contribute toward the formulation based on natural products is inarguably their low or absent toxicity, their complete biodegradability, the

availability from renewable sources, and in most cases and situations, the undoubted leverage displayed by their low-cost if compared with those compounds obtained by total chemical synthesis (Tringali, 2001)

Chitosan is a linear β-1,4-$_D$-glucosamine is a biocompatible, non toxic compound mainly obtained by deacetylation of chitin, a natural structural component present for instance in crustaceans.

Chitosan inherent biocide properties which is excellent in against wide range of microorganisms such as filamentous fungi, yeast and bacteria (Coma, Deshcamps, & Martial- Gros, 2003; Moller, Grelier, Pardon, & Coma, 2004). This biopolymer also presents interesting properties by excellent film forming capacity and gas and aroma barrier properties at dry conditions, which makes it a suitable material for designing food packaging structure (Caner, 2005).

Unfortunately, the hygroscopic properties of the bio-packaging containing polysaccharides are responsible for their weak moisture barrier and thus have little or no influence on the dehydration/rehydration phenomena of the foodstuffs, a property crucial for maintaining organoleptic and microbiological food qualities (F. Se´bastien et al, 2006).

Therefore, the biopolymer need to associate with the polysaccharide with a more moisture- resistant polymer, while maintaining the overall biodegradability of the product. The association with polylactic acid being considered in the form of (blending) films (F. Se´bastien et al, 2006).

In 1996, the outbreak of food-borne illness by Escherichia coli O157:H7 in Japan was intensely influenced to strengthen the inspection of the imported meats in Korea, and also to make every efforts to seek promptly out an infected person who suffering from diarrhea. The chemical components of corn silk, which has been used as a diuretic in Chinese medicine, were analyzed and the antibacterial activity against Escherichia coli O157:H7 (*H. J. Chung et al*, 2003)

Throughout the years, many research have been done to improve the performance of biodegradable film in the food packaging area. From all the points above, an attempt is done to investigate the effect of different chitosan concentration in a composite biodegradable film.

1.4 Objectives

The objectives of this study are:

a) To fabricate composite of chitosan and corn silk.
b) To characterize composite of chitosan and corn silk.

1.5 Scope of study

The scope of this study are:

a) The fabrication of biodegradable film using chitosan and corn silk.
b) The characterization of biodegradable film using Atomic Force Microscope (AFM) , Fourier Transform Infrared (FTIR) Spectroscopy, Differential Scanning Calorimetry (DSC) and Thermogravimetric Analysis (TGA).

CHAPTER 2

LITERATURE REVIEW

2.1 Biopolymers

Bio-based polymers may be divided into three main categories based on their origin and production. It depend on the sources of biopolymer. Some of biopolymer being extracted or removed from biomas. Examples are polysaccharides such as starch and cellulose and proteins like casein and gluten. The extraction process involved is the process to isolate the biopolymer from certain structure such as from corn silk, husk, empty fruit bunch, wheat straw, or fat from meat and protein from bone. Hydrolysis is one of the extraction process involved by remove water and certain chemical compound in the sources. (Tharanathan, 2003)

Secondly is the polymer produced by classical chemical synthesis using renewable bio based monomer The polymer is the chain of monomer. So , the bio-based monomer will bind to one another to form biopolymer. The good example of it is polylactic acid, a bio-polyester polymerized from lactic acid monomers. The monomers themselves may be produced via fermentation of carbohydrate feedstock. (Petersen *et al.*, 1999)

Finally, the polymer produced by microorganisms or genetically modified by bacteria such as polyhydroxylalkonoates. The developments with bacterial cellulose however are in progress because of this polymer is still in research. (Petersen *et al.*, 1999)

The most common bio-based polymers, materials and packaging are presented in the following (Petersen *et al.*, 1999)

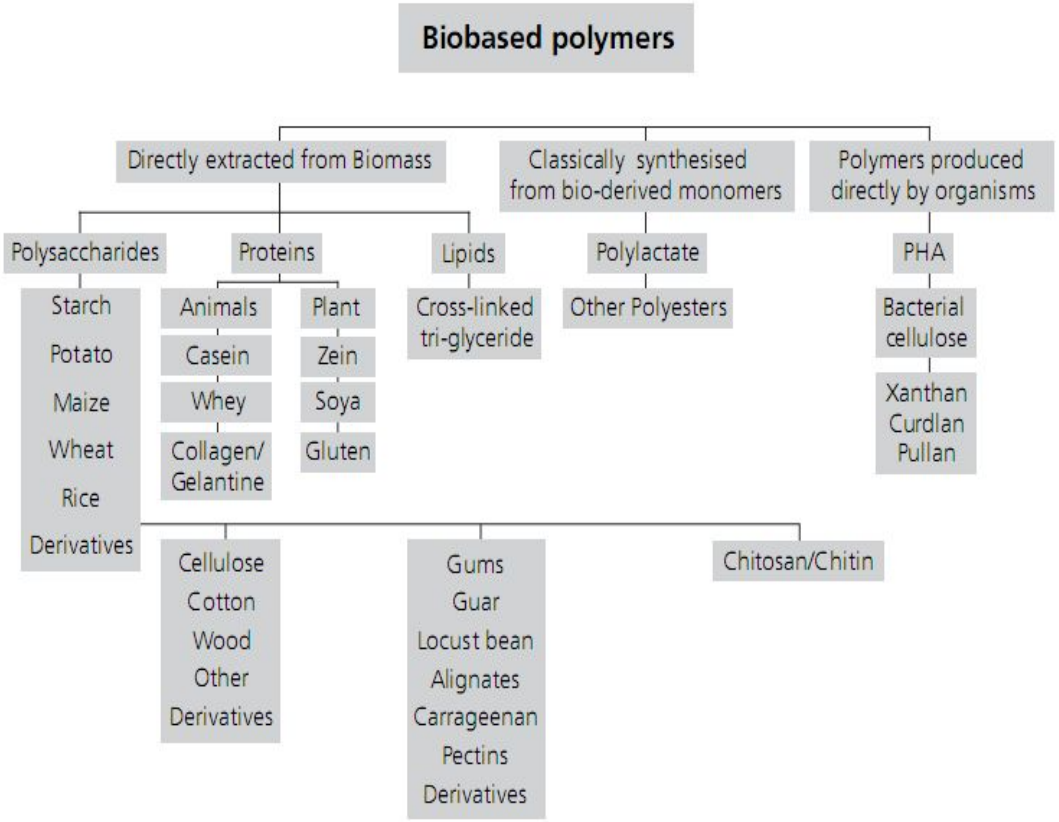

Figure 2.1: Schematic presentation of bio-based polymers based on their origin and method of production. Source: Adapted from Petersen *et al.*, 1999.

2.2 Biodegradable films

Biodegradable film is the biopolymer films. It is environmentally friendly and could be degraded by microorganisms without further assistance. The films may contain of polysaccharides, proteins, lipids and etc. The films easy to degrade due to it's the polymer that built in it is made of organic materials. The biodegradable may consist of one or two organic materials. If the films consist of two organic materials that can be easily biodegrade, it called as biocomposite degradable films. (Tharanathan, 2003)

The barrier properties of the biodegradable films are important parameters when considering a suitable barrier in foods and food packaging. Protein and polysaccharide films are generally good barriers against oxygen at low and intermediate relative humidity (RH) and, while having relatively good mechanical properties, most of them have poor moisture barrier properties (Lai and Padua., 1998).

Films based on biopolymers are generally sensitive to the relative humidity of the air since they are normally hygroscopic and have limited mechanical resistance compared with synthetic films. Nevertheless protein-based films display high deformability. A possible solution to improve the mechanical characteristics of protein-based films could be the mixing of these biopolymers with synthetic polymers (Tharanathan, 2003)

2.3 Biocomposite Films

Composites are engineered materials made from two or more constituents with significantly different physical or chemical properties from their components, which remain separate and distinct within the finished structure. There are two types of constituent materials, which are known as matrix and reinforcement components. The matrix in the material surrounds and supports the reinforcement component by maintaining their relative positions. (Tharanathan, 2003)

Most of the literature published on composites deals with synthetic polymers. The sources are plant cell walls or some types of living organisms if it is not in conflict with ethical principles. Polysaccharides are environmentally friendly and could be degraded by microorganisms without further assistance. It is believed that their use could be profitable especially when they are by-products of other processes. As there are other natural polymers or related carbohydrate groups like proteins, lignin or cyclodextrins, which are used as constituents of polysaccharide-containing composites, their environmental impact is also covered. (Tharanathan, 2003)

There were many research been run such as by combining the polysaccharides from rice starch and chitosan or by combining the protein from whey protein and zein corn protein. Films based on biopolymers are generally sensitive to the relative humidity of the air since they are normally hygroscopic and have limited mechanical resistance compared with synthetic films. Nevertheless protein-based films display high deformability. A possible solution to improve the mechanical characteristics of protein-based films could be the mixing of these biopolymers with synthetic polymers (Tharanathan, 2003),

2.4 Polysaccharides

Carbohydrates (Polysaccharides) are naturally occurring compounds of carbon, hydrogen and oxygen. Many carbohydrates have the empirical formula CH_2O. One distinct example is the molecular formula for glucose which is $C_6H_{12}O$. All carbohydrates vary dramatically in their properties such as the case of sucrose, also known as table sugar, and cotton that are both carbohydrates but displaying distinguishable characteristics. The monosaccharide, often called simple sugars, are the simplest carbohydrate units as they cannot be further hydrolyzed to smaller carbohydrate molecules (Fessenden et al., 1998).

The monomer of polysaccharides which monosaccharide has many structure or isomer. D-glucose, D-fructose, D-galactose, D-ribose and 2-deoxy-D-ribose is five from many isomer of monosaccharide.

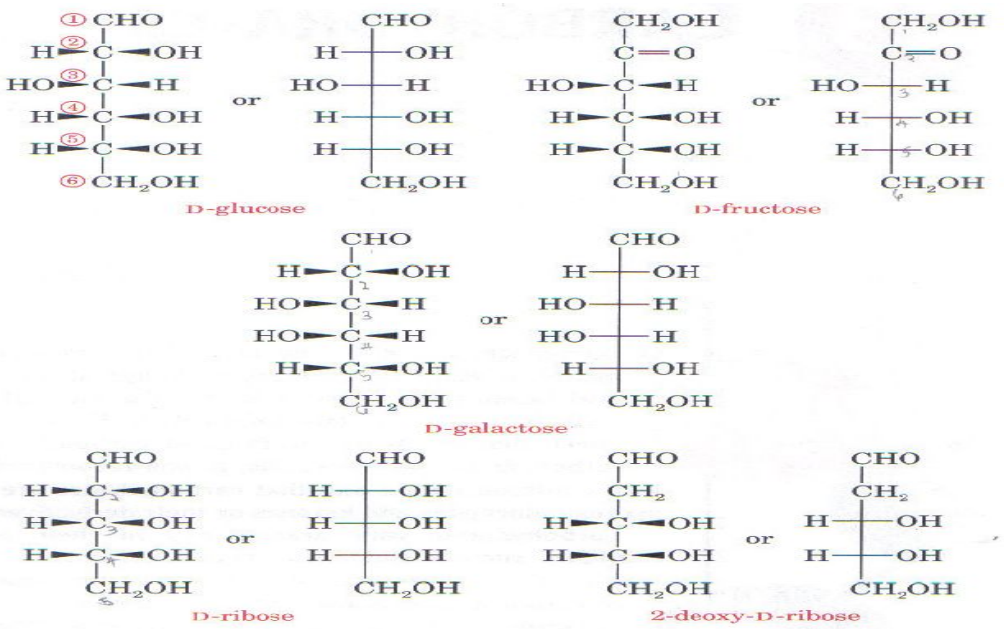

Figure 2.2: Some important monosaccharides (Fessenden et al., 1998).

Monosaccharides also known to be able to bond together to form dimers, trimers, etc. and ultimately, polymers. Polysaccharides that are composed of two to eight units of monosaccharide are referred to as oligosaccharides (Greek oligo-, “a few”). If more than eight units of monosaccharide result from the hydrolysis process, the carbohydrate is a polysaccharide. Common examples of carbohydrates are starch found in rice, flour and corn-starch, cellulose, a fibrous constituent of plants and the principal component of cotton and chitin which can be found mostly in arthropods like crabs and insects (Fessenden et al., 1998).

2.5 Chitosan

The principal structural polysaccharide of the arthropods like crabs, shrimps and insects is chitin. It occurs as an important constituent of the exoskeleton of many organisms, particularly crustaceans, insects and molluscs and in the walls of most fungi and some algae (Poirier and Charlet, 2002).

It has been estimated that 10^9 tons of chitin are biosynthesized each year. Chitin is a linear polysaccharide consisting of β-linked N-acetyl-D-glucosamine. Upon hydrolysis, chitin yields 2-amino-2-deoxy-D-glucose in which the acetyl group is lost in the hydrolysis step. In nature, chitins are bonded to non-polysaccharide materials such as proteins and lipids (Fessenden et al., 1998).

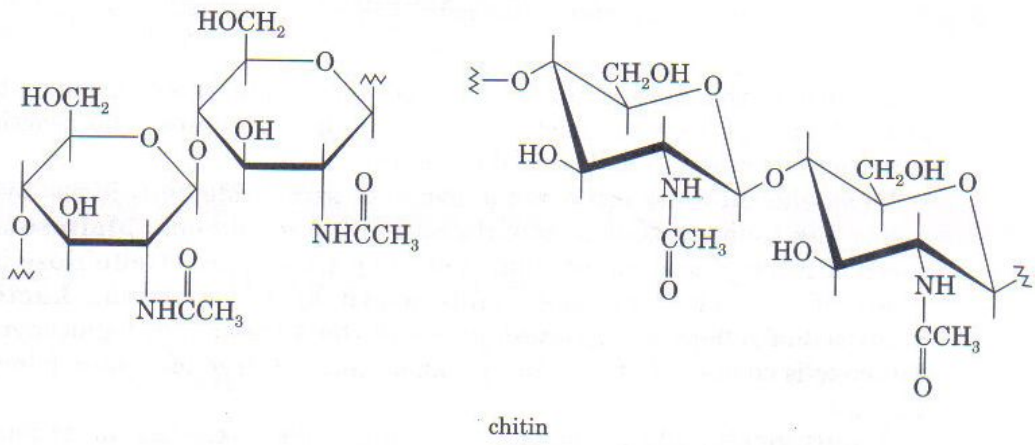

Figure 2.3: Structural formula of chitin. Source: Fessenden et al., 1998

Chitin is chemically identical to cellulose, except that the secondary hydroxyl group on the alpha carbon atom of the cellulose molecule is substituted with acetoamide groups (Figure 4).

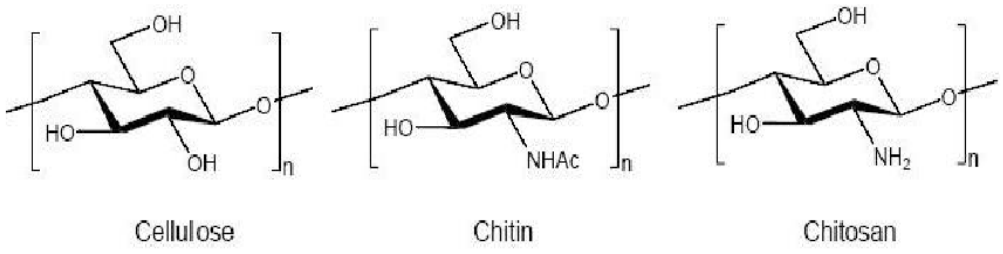

Figure 2.4: Comparing the chemical structures of cellulose, chitin and chitosan. Source: Fessenden et al., 1998

Also, according to research, chitin, a homopolymer of b-(1 → 4)-linked N-acetyl-D-glucosamine, is one of the most abundant, easily obtained, and renewable natural polymers, second only to cellulose (Tolaimate *et al.*, 2000). A sharp nomenclature with respect to the degree of N-deacetylation has not been defined

between chitin and chitosan. In general, chitin with a degree of deacetylation of above 70% is considered as chitosan.

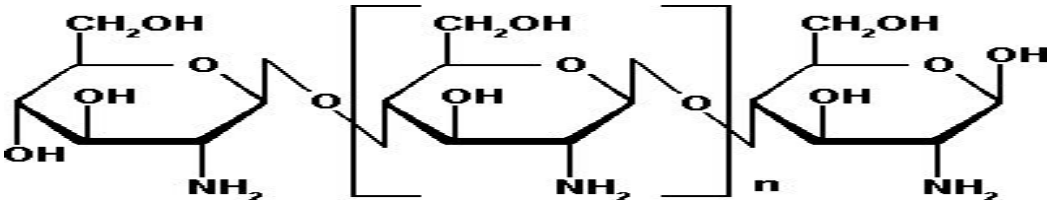

Figure 2.5: A chemical formula of chitosan in Haworth´s projection

Chitosan is produced by thermochemical alkaline deacetylation of chitin. It is a biopolymer with unique properties favorable for a broad variety of industrial and biomedical applications. Chitosan is characterized by its degree of N-acetylation (DA) and this degree influences not only its physicochemical characteristics but also its biodegradability and immunological activity. (Tolaimate et al., 2000).

It is worth noting that chitosan derived from b-chitin shows higher reactivity than that derived from a-chitin in N-phthaloylation (Tolaimate et al., 2000).

Chitosan possesses repeating units of 1,4 linked 2-deoxy-2-aminoglucose. The amino group NH2 can be protonated to NH3+ and readily form electrostatic interactions with anionic groups in an acid environment. This property has been applied on edible films (Xu et al., 2005).

2.6 Starch

Starch is the second most abundant polysaccharide. It is also worth noted that it can be found as a reserve polysaccharide present in the endosperm of the grain of corn (Zea mays L.), banana pulp (Musa paradisiaca), yucca (Manihot esculenta) and among others (Romero-Bastida et al., 2005).

Most importantly, starch is the main source of carbohydrate in the human diet and contributes as a valuable ingredient to the food industry where it is being widely used as a thickener, gelling agent, bulking agent and water retention agent. In most cases, natural starch is partially crystalline in its native granule form (Iida et al., 2008).

Starch can be separated into two principal fractions based upon solubility when triturated, meaning literally pulverized, with hot water. About 20% of starch is amylose which is soluble and the remaining 80% is amylopectin which is widely insoluble (Fessenden et al., 1998).

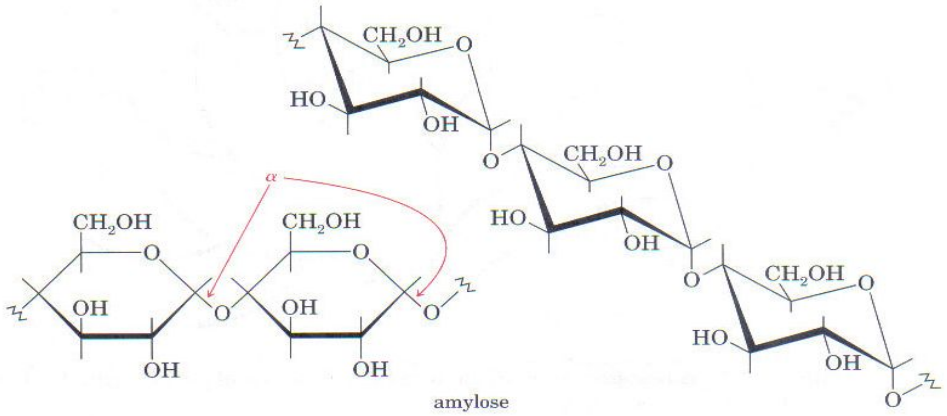

Figure 2.6: A structural formula of amylose (Fessenden et al., 1998)

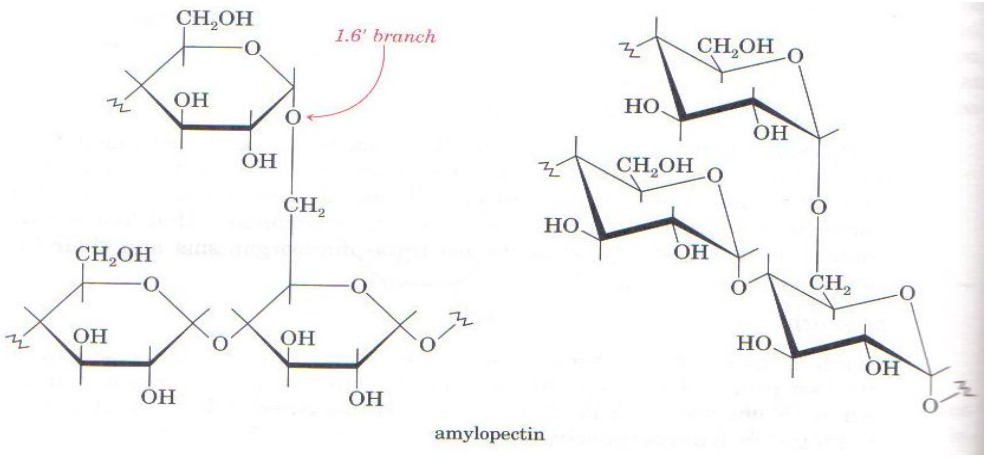

Figure 2.7: A structural formula of amylopectin (Fessenden et al., 1998

The amylose fraction is essentially linear, whereas amylopectin is highly branched. The two starch components have different properties and are not suitable for the same applications (Stawski, 2008; Paes et al., 2008) and amylose is the constituent responsible for the film-forming capacity of starches (Romero et al., 2005).

The amount of both starch components has been influential on many various properties of these materials such as swelling capacity, water solubility, water-binding capacity (Sandhu et al., 2005), barrier and mechanical properties of starch films (Rindlav et al., 1998) and microscopic properties.

There are 250 or more glucose units per amylose molecule and the exact number depends upon the species of animal or plant. In the measurement of chain length, it is complicated by the fact that natural amylose degrades into smaller chains upon separation and purification. Amylose molecules form helices or coils around I2 molecules. (Fessenden et al., 1998).

Many starch-based ingredients that have been developed are principally used to take up water and to produce viscous fluids and/or gels to impart the desired textural quality to food products. In addition, water immobilization in foods prevents growth of micro-organisms, thereby contributing to a longer shelf life (Ritota et al., 2008). Gelatinization is the process where change the linear amylase to become enriched with amylopectin.

Very often, the functional properties of starch are acquired by gelatinization, which comprises of heating a suspension of granules above a characteristic temperature, causing the starch granule to swell irreversibly (Marques et al., 2006). During the swelling, the linear amylose molecules diffuse out of the swollen granules and are preferentially solubilized. The final state can be viewed as a continuous phase of amylose in which more or less overlapped swollen granules, enriched with amylopectin, are suspended.

All of the current understandings of gelatinization are based on heating starch granules by using conduction heating modes. Under these conditions, gelatinization is understood as the cumulative irreversible changes that occur to a starch granule in the presence of moisture and heat (Palav and Seetharaman, 2006). These changes again as discussed above, include granule swelling due to absorption of moisture in the amorphous regions of the granule, leaching of small molecular weight polymers including amylose, loss of the crystalline order and the consequent loss of birefringence, leaching of larger molecular weight polymers from the granule including fragments of amylopectin and, finally, starch solubilization (Sakonidou et al., 2003).

On heating starch in excess water conditions, the granules are also found to swell in which the starch polymers are partially solubilized and leached from the granules, and finally the starch granules disintegrate (Bilbao et al., 2008). Starch gives two distinct endotherms during gelatinization at low water contents. According to (Donovan et al., 2004) that these endotherms were due to phase transitions, which were governed by the degree to which ordered regions within granules were hydrated. The high temperature transition is due to melting of crystallites without adequate moisture.

When excess water is present, the high temperature transition disappears. Further reports by Donovan suggest that, at high water contents, the amorphous regions of the granules imbibed water and swelled, resulting in stripping or separation of starch chains from portions of these crystallites. When all crystals are stripped at high moisture levels, there would not be any crystallites remaining to be melted at high temperature (Ratnayake and Jackson, 2007)

2.7 Corn Silk

Corn is one of the most widely planted crops in the world. It is estimated that the total yield of corn in 2001 was 6.1×10^8 Mt. Only in China, the yield of corn may have reached 1.2 × 108 Mt.(Chao et. al, 2003).

Cornsilk has detoxifying, relaxing and diuretic activity. Cornsilk is used to treat infections of the urinary and genital system, such as cystitis, prostatitis and urethritis. Cornsilk helps to reduce frequent urination caused by irritation of the bladder and is used to treat bed wetting problems. In China, cornsilk is traditionally used to treat oedema and jaundice. Studies indicate that cornsilk can reduces blood clotting time and reduce high blood pressure.(Chung et al, 2003)

There was much kind of protein and minerals in corn silk. The research is still going on, but it is believe that corn silk maybe also contain corn protein which is zein. Zein normally produced commercially from corn gluten meal (CGM). CGM is a co-product material that is obtained during starch production. It is low priced and is used mainly as animal feed (Ghanbarzadeh, Oromiehi and Musavi., 2006). Zein forms films with high tensile strength and low water vapor permeabilities compared to other protein-based films. It also has a desirable heat seal property. Furthermore, corn zein can be used as a binder to fatty acids as both are alcohol soluble. Zein is insoluble in water. The insolubility of zein in water is due to the low content of polar amino acids and a high content of nonpolar amino acids

2.8 Related Research Biocomposite Films

Numerous investigations have been reported on the studies of films made from chitosan (Park et al., 2002, and Wiles et al., 2000) and chitosan blends with natural polymers (Lazaridou and Biliaderis, 2002 and Xu et al., 2005)

Chitosan provides unique functional, nutritional, and biomedical properties, and its present and potential uses range from dietary fiber to a functional ingredient

and processing aid. Some of the well known applications of chitosan include its use for prevention of water pollution, medicine against hypertension, antimicrobial and hypocholesterolemic activity, flavor encapsulation, seed coating, film-forming, and controlled release of food ingredients and drugs (Struszczyk and Pospieszny, 1997).

Rice starch and its major components, amylose and amylopectin, are biopolymers, which are attractive raw materials for use as barriers in packaging materials. They have been used to produce biodegradable films to partially or entirely replace plastic polymers because of its low cost and renewability, as well as possessing good mechanical properties (Xu, Kim, Hanna, and Nag, 2005). However, wide application of starch film is limited by its mechanical properties and efficient barrier against low polarity compounds (Azeredo et al., 2000 and Kester and Fennema, 1986). This constraint has led to the development of the improved properties of rice-based films by modifying its starch properties and/or incorporating other materials. (Films based on biopolymers are generally sensitive to the relative humidity of the air since they are normally hygroscopic and have limited mechanical resistance compared with synthetic films.

Nevertheless protein-based films display high deformability. A possible solution to improve the mechanical characteristics of protein-based films could be the mixing of these biopolymers with synthetic polymers (Tharanathan, 2003), such as poly(vinyl alcohol) (PVA), which is also hydrophilic and biodegradable. Some studies on the development and characterization of films based on PVA and protein blends have been published, such as PVA/wheat (Dicharry et al., 2006), PVA/collagen hydrolysate (Alexy et al., 2003), and PVA/gelatin (Bergo et al., 2006) have demonstrated the presumed biodegradability of these blended films.

2.9 Polyethylene glycol impact in composite biofilms

The composite biofilms of chitosan-polylactic acid incorporated with Polyethylene glycol will make the product more flexible and ‘easy to recover’

materials. The chitosan-polylactic acid films will have variable thickness depend on their blending mixture and the concentration of polyethylene glycol. The higher the polyethylene glycol, the easier the films of chitosan were removed from polypropylene support and more flexible there were (F. Se'bastian et al., 2006).

The higher concentration of polyethylene glycol in the composite films, the more higher the water vapor transmission rate will be, it is due to progressive film plasticization which is associated with modification of the hydrophilic character of polylactic acid film. The polyethylene glycol, thus decrease the material cohesion by creating intermolecular spaces and increasing water molecule diffusion coefficient or the easier separation of polyethylene glycol with the amorphous phase of polylactic acid which significantly explain the result (Renouf-Glauser et al., 2005).

The content of polyethylene glycol will also reduce the rigidity and the brittleness of materials, thus improving their mechanical properties and their recovery. It is due to hydrolytic reactions by water absortion(Renoulf-Glauser et al., 2005).

2.10 Biological Properties

Mycotoxins can be characterized as secondary metabolites of various toxigenic fungi. Mycotoxins occur in a range of human and animal disease (Coker, 1997). Exposure to mycotoxins can produce both acute and chronic toxicities ranging from death to deleterious effects upon.

The development of active materials based on antifungal coatings and films could be one solution to limiting the growth of these phytopathogens. Chitosan used as polymeric matrix to produce films from renewable resources which exhibit potential antifungal properties on mycotoxinogen strains because of its good film forming properties and its recognized antimicrobial activity (Coma et al., 2002)

The used of chitosan as antifungal activities being prove by coating delayed Alternaria sp, Penicilliium sp and Cladosporium sp growth. This behavior is similar to the action of synthetic preservatives such as calcium propionate and potassium sorbate (Agullo et al., 2003).

The polycationic property allows chitosan to interact with negatively charged substance, thereby exhibiting antimicrobial activity on molds. Thus, it show that the limitation of the development of mycotoxinogen fungi (Agullo et al., 2003).

Three mechanisms have been proposed for chitosan's antifungal properties. The biopolymer appears work both by iinterfering directly with fungal growth and also on plants, by activating many defense rensponses. The chelating properties of chitosan make it an antifungal agent. Moreover, a strong inhibiton of Aspergillus niger spore germination on a solid medium supplemented with chitosan and observe that chitosan produced spore aggregation and morphological anomalies. In previous study, the analysis through epifluorescence show that chitosan may acts on nucleic acids (Sebti et al., 2005).

2.11 Biodegradable Film Preparation

A composite film formulation can be tailored made to suit to the needs of a specific commodity or farm produce. For example, oranges having a thick peel are prone to anaerobic conditions, which lead to an early senescence and spoilage if the composite film is rich in lipids. Phase separation encountered during the preparation of composites is overcome by using emulsifying agents. Use of plasticizers such as glycerin, enthylene glycol, sorbitol, etc. in the film formulations or composites is advantageous to impart pliability and flexibility, which improves handling (Garcia et al., 2000). Use of plasticizers reduces the brittleness of the film by interfering with the hydrogen bonding between the lipid and hydrocolloid molecules.

The use of wax coating of fruits by dipping is one of the age-old methods that were in vogue in the early 12th century (Krochta et al., 1994). This was practiced in China, essentially to retard water transpiration losses in lemon and oranges. Later fat coating of food products, specifically called ‘‘larding’’ was in vogue in England. Sausage casing used very commonly nowadays is nothing but a material derived from a protein source (gelatin). Usually a film thickness of ~2.5 mm is employed, and coating is done by several methods. Films are reformed thin membranous structures, which are used after being formed separately, whereas in coatings the thin film is formed directly on the commodity.

The coating can also be done by a foam application method. Emulsions are usually applied by this method. In here extensive tumbling action is necessary to break the foam for uniform distribution of the coating solution, over the commodity surface. Coating by spraying is the conventional method generally used in most of the cases. Due to high pressure (60–80 psi) less coating solution is required to give a better coverage. Programmable spray systems are available for automation during such operations.

Biodegradable packaging films are generally prepared by wet casting of the aqueous solution on a suitable base material and later drying. Choice of the base material is important to obtain films, which can be easily removed without any tearing and wrinkling. Infrared drying chambers are advantageous in that they hasten the drying process (Tharanathan, Srinivasa, and Ramesh, 2002). Optimum moisture content (~5–8%) is desirable in the dried film for its easy peel off from one edge of the base material.

2.12 Characterization of Biodegradable Film

There are several methods for characterize the biodegradable films which are using Atomic Force Microscopy (AFM), Fourier Transform Infrared (FTIR), Differential Scanning Calorimeter (DSC), Thermo Gravitation Analyzer (TGA) and last but not least Scanning electron microscope (SEM).

2.12.1 Atomic Force Microscopy (AFM)

The atomic force microscope (AFM) is one of the most powerful tools for determining the surface topography of native biomolecules at subnanometer resolution. Unlike X-ray crystallography and electron microscopy (EM), the AFM allows biomolecules to be imaged not only under physiological conditions, but also while biological processes are at work. Because of the high signal-to-noise (S/N) ratio, the detailed topological information is not restricted to crystalline specimens. Hence single biomolecules without inherent symmetry can be directly monitored in their native environment.

The AFM can also provide insight into the binding properties of biological systems. In order to determine the specific interaction between two kinds of molecules in an AFM, one kind is bound to the tip of a cantilever and the other kind covers the surface of the sample support. The adhesion force upon separation is then a measure of the binding strength. This method allowed the intermolecular forces between individual ligand-receptor pairs, complementary DNA strands, cell adhesion proteoglycans, and the specific antigen-antibody interaction to be determined.

The AFM works in the same way as our fingers which touch and probe the environment when we cannot see it. By using a finger to "visualize" an object, our brain is able to deduce its topography while touching it. The resolution we can get by this method is determined by the radius of the fingertip. To achieve atomic scale resolution, a sharp stylus (radius ~1-2 nm) attached to a cantilever is used in the

AFM to scan an object point by point and contouring it while a constant small force is applied to the stylus. With the AFM the role of the brain is taken over by a computer, while scanning the stylus is accomplished by a piezoelectric tube. This simple technique provides a peep into the microscopic world, and it enables us to understand how the system of biological systems might function.

Atomic Force Microscopy (AFM) is rather new method to characterize the surface of biodegradable film (Bining *et al.*, 1986). A sharp tip with a diameter smaller than 100 A is scanning across a surface with a constant force. London –van der Walls interactions will occur between the atoms in the tip and the surface and these forces are detected. This will result in a line scan or profile of the surfaces (Mulder, 1996). It has emerged as the technique of choice for conducting single molecule force spectroscopy, owing largely to the limited range of forces that can be applied by competing methodologies such as the use of optical tweezers or magnetic beads (Strick *et al.*, 2003). The level of information obtained from AFM images, however, depends critically on the size, shape, and terminal functionality of the probe tips used for imaging and whether the sample consists of isolated molecules or packed molecular arrays (Engel *et al.*, 1997)

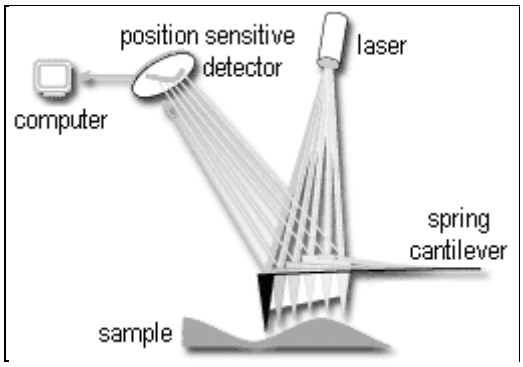

Figure 2.8: A layout of an AFM operation

Figure 2.9: Atomic Force Microscope Machine

2.12.2 Fourier Transform Infrared (FTIR)

Fourier Transform Infrared Spectroscopy (FTIR) is most useful for identifying chemicals that are either organic or inorganic. It can be utilized to quantitate some components of an unknown mixture. It can be applied to the analysis of solids, liquids, and gasses. The term Fourier Transform Infrared Spectroscopy (FTIR) refers to a fairly recent development in the manner in which the data is collected and converted from an interference pattern to a spectrum. Today's FTIR instruments are computerized which makes them faster and more sensitive than the older dispersive instruments. (Kim et al., 2004).

FTIR can be used to identify chemicals from spills, paints, polymers, coatings, drugs, and contaminants. FTIR is perhaps the most powerful tool for identifying types of chemical bonds (functional groups). The wavelength of light absorbed is characteristic of the chemical bond as can be seen in this annotated spectrum. (Kim et al., 2004).

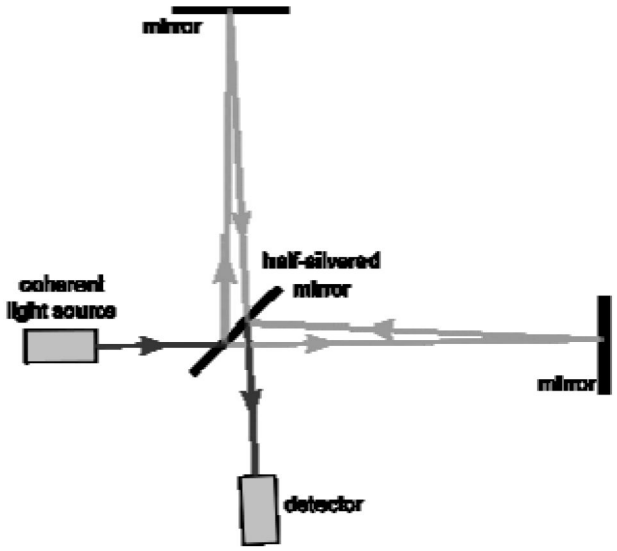

Figure 2.10: The outline of the FTIR process

By interpreting the infrared absorption spectrum, the chemical bonds in a molecule can be determined. FTIR spectra of pure compounds are generally so unique that they are like a molecular "fingerprint". While organic compounds have very rich, detailed spectra, inorganic compounds are usually much simpler. For most common materials, the spectrum of an unknown can be identified by comparison to a library of known compounds. WCAS has several infrared spectral libraries including on-line computer libraries. To identify less common materials, IR will need to be combined with nuclear magnetic resonance, mass spectrometry, emission spectroscopy, X-ray diffraction, and/or other techniques. (Skoog et al., 2003)

Because the strength of the absorption is proportional to the concentration, FTIR can be used for some quantitative analyses. Usually these are rather simple types of tests in the concentration range of a few ppm up to the percent level. For example, EPA test methods 418.1 and 413.2 measure the C-H absorption for either petroleum or total hydrocarbons. The amount of silica trapped on an industrial hygiene filter is determined by FTIR using NIOSH method 7602.(Skoog et al., 2003)

The Fourier-transform infrared spectrometer gives an absorbance spectrum that can detect much higher absorbances than the UV-visible spectrometer. Here is a long-winded explanation of why, which might also help you understand the lecture portion of the class. The FTIR acquires an absorbance spectrum without using a